IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant: Rajiv SHAH, et al.

Title: METHOD FOR FORMULATING A GLUCOSE OXIDASE ENZYME WITH A DESIRED PROPERTY OR PROPERTIES AND A GLUCOSE OXIDASE ENZYME WITH THE DESIRED PROPERTY

Appl. No.: 10/715,143

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Examiner: Yong D. Pak

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APPEAL BRIEF UNDER 37 C.F.R. § 41.37

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Sir:

Under the provisions of 37 C.F.R. § 41.37, this Appeal Brief is being filed with the appropriate appeal fee under 37 C.F.R. 41.20(b)(2). An appeal fee of $500 is being submitted with Applicant's Appeal Brief together with a credit card payment form in the amount of $500.00 covering the 37 C.F.R. 41.20(b)(2) appeal fee. If this fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

This communication is an Appeal Brief, responsive to the Final Office Action dated January 16, 2007, concerning the above-referenced patent application.

I. REAL PARTY IN INTEREST

The real party in interest for the above referenced patent application and the present Appeal is the assignee of record for the above referenced patent application, Medtronic Minimed, Inc., as recorded at Reel 012818, Frame 0025.

II. RELATED APPEALS AND INTERFERENCES

Applicant is not aware of any interferences or legal proceedings that would have a bearing on the Board's decision in the present Appeal.

The present application is a Divisional application of U.S. Application No. 10/035,918. An appeal brief has been filed for U.S. Application No. 10/035,918 dated April 02, 2007, no decision has been rendered in that Appeal.

The present application also claims the priority filing date of U.S. Provisional Application No. 60/335,585 (now expired), for which no substantive examination on the merits was conducted by the U.S. Patent and Trademark Office.

III. STATUS OF CLAIMS

Claims 1-17 are pending in the application. However, claim 18 has been withdrawn from consideration by the Examiner. Accordingly, claims 1-17 are pending and under consideration in the present application. Each of those claims is included in at least one of the rejections under general grounds identified in the Final Office Action and discussed in Sections VI. and VII., below. The present appeal relates to each of the rejections and, thus all of the rejected claims (i.e., claims 1-17).

IV. STATUS OF AMENDMENTS

No amendments have been filed, subsequent to the Final Office Action of January 16, 2007.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Embodiments of the present invention relate, generally, to a method employing directed evolution techniques for formulating a glucose oxidase enzyme having peroxide-resistant characteristics for use, by way of example, in a sensing device.

An example implantable sensing system contains a sensing device that is inserted into a vein, an artery, or any other part of a human body where it could sense a desired parameter of the implant environment. An enzyme may be placed inside of the sensing device and employed for sensing. If the device is a glucose-sensing device, then a combination of glucose oxidase (GOx) and human serum albumin (HSA) may be utilized to form a sensor protein. During operation in a sensing device, glucose oxidase reacts with oxygen and oxidizes. The oxidation of glucose oxidase results in the formation of a hydroperoxy adduct, which transforms into hydrogen peroxide.

The applicant has recognized that, an obstacle to creating sensors that are long-lived and stable over time has been that glucose oxidase, when immobilized (e.g., for use in a sensor), undergoes oxidative inactivation by the aforementioned hydrogen peroxide over time. Since the lifetime of glucose sensors primarily depends on the lifetime of glucose oxidase, the effects of the peroxide on the glucose oxidase can severely limit the lifetimes of glucose sensors.

Prior processes for addressing the peroxide degradation of glucose oxidase have involved the use of additives or neutralizing agents for deactivating, removing or neutralizing peroxide. (Examples of such prior art are discussed below with respect to the Valdes et al. reference, the Stemmer patent, the Hatzinikolaou et al. article, and the Wagner et al. patent). Embodiments of the present invention relate to an unexpected change in direction of the state of the art by employing directed evolution techniques to formulate a glucose oxidase gene having desired peroxide resistant properties.

Evolution under non-stress circumstances takes years. Accordingly, evolution may be manipulated in embodiments of the invention for specific enzymatic functions. In embodiments

of the invention, a technique known as directed evolution is employed to evolve glucose oxidase, to formulate a glucose oxidase that possesses improved resistance to peroxide. A glucose oxidase formulated pursuant to embodiments of the present invention may improve the longevity of a biosensor in which it is employed.

According to the claims under appeal, a method comprises formulating an enzyme by obtaining an organism with a glucose oxidase gene. Using that organism growing multiple colonies of the organism. Next altering the environment of the colonies and screening the colonies to identify colonies with active glucose oxidase.

The multiple colonies are then screened for desirable peroxide resistant properties. The colonies are screened by determining whether the colonies contain active glucose oxidase and determining whether the colonies have desired peroxide resistant properties. Determining whether the colonies have desired peroxide resistant properties involves incubating the colonies in peroxide and determining whether the colonies have active glucose oxidase after incubating, including measuring a concentration of the glucose oxidase.

One embodiment of the invention involves, for example, a library of organisms, all of which contain glucose oxidase. In one embodiment, this library of organisms is grown in separate colonies with a conventional growth medium. In this embodiment, the environment of each colony is subsequently altered. For example, the environment of each colony may be altered by introducing peroxide to it. A screening procedure may be employed after the environments of the colonies have been altered. The screening procedure may involve processes of determining which of the colonies contain active glucose oxidase. Those colonies that still contain active glucose oxidase after their environments have been altered may possess desirable peroxide resistant qualities. Glucose oxidase from those colonies still containing active glucose oxidase may be tested for functionality, for example, by immobilizing the glucose oxidase in a sensor. In other embodiments of the invention, following at least a portion of the screening procedure, the environments of the colonies may be altered another time if desired. For example,

in one embodiment, altering the environments of the colonies by adding more peroxide may reduce the number of colonies that proceed to the functionality testing.

Those colonies that contain active glucose oxidase after the alteration of their environments and incubation procedures may possess desirable peroxide resistant qualities. Glucose oxidase from those colonies containing active glucose oxidase may be tested for functionality, for example, by immobilizing the glucose oxidase in a sensor. In other embodiments of the invention, following at least a portion of the screening procedure, the environments of the colonies may be altered another time if desired, for example, by adding more peroxide.

The method recited in the pending claims of the present application can provide significant advantages over the prior art of record. The ability to form a stable enzyme which is peroxide resistant and which may be employed in an altered environment (oxygen free environment), such as a biosensor, can provide significant advantages in extending the life of biosensors. When used in an implanted medical device (such as an implanted blood glucose sensor), peroxide resistance and, thus, a capability for extending the life of the enzyme can provide considerable patient comfort and safety advances, for example, by reducing the frequency of surgical sensor replacements. Moreover, the ability to form enzymes with peroxide resistant properties suitable for biosensor applications in a relatively inexpensive, non-complicated and reliable process can provide significant advantages with respect to the ability to manufacture readily available supplies of the enzyme and, thus, increasing the availability of longer-life biosensors to more patients.

By a method in accordance with embodiments of the present invention, a glucose oxidase enzyme may be formulated to exhibit desired peroxide resistant properties. As such, further additives or other mechanisms for deactivating, removing or neutralizing peroxide may not be required. Thus, the disclosed method involves a distinct departure from the conventional direction of those skilled in the art.

Claim 1	Specification
A method for formulating an enzyme comprising:	Title; pg. 1, ll. 21-24; pg. 4, ll. 10-22; and pg. 7, ll. 11-12.
obtaining an organism with a glucose oxidase gene;	Pg. 5, ll. 14-16; pg. 8, ll. 6-13; Fig. 2, ref. 12; pg. 14, ll. 1-8.
growing multiple colonies of the organism;	Pg. 5, l. 17-18; pg. 10, ll. 1-9; Fig. 2, ref. 18.
altering the environment of the colonies	Pg. 6, l. 16 – 17; pg. 14 l. 9-10; Fig. 4, ref. 46.
screening the colonies to identify colonies with active glucose oxidase after altering the environment of the colonies.	Pg. 5, ll. 18-21; pg. 10, l. 10 to pg. 13, l. 15; pg. 14, ll. 15-20; Fig. 2, ref. 20.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Claims 1-17 are rejected, as follows:

1. Claims 7-10 and claims 11-18 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

2. Claims 1-3 and 7-8 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Valdes et al., Hatzinikolaou et al. and Stemmer.

3. Claims 4-6 and 9-17 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Valdes et al., Stemmer and Hatzinikolaou et al. as applied to claims 1-3 and 7-8 above, and further in view of Wagner and Aldrich Catalog.

As noted in Section III, above, the present appeal relates to each of the above rejections and, thus, all of the rejected claims (i.e., claims 1-17).

VII. ARGUMENT

1. Appeal Of Rejection Of Claims 7-10 and 11-18 Under 35 U.S.C. § 112, Second Paragraph

Claims 7-10 and 11-18 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is respectfully traversed. Applicant requests that the rejection be reversed and the rejected claims allowed in view of the following remarks.

In explaining the rejection the Examiner stated:

> Claims 7-10 recite the phrase "predefined, desired functionality". The metes and bounds of this phrase in the context of the above claims are not clear to the Examiner. A perusal of the specification did not provide the Examiner with a specific definition for the above phrase. Therefore, it is not clear to the Examiner either from the specification or from the claims as to what specific "functions" of glucose oxidases are encompassed in the phrase "predefined, desired functionalities".

This rejection is respectfully traversed. In particular, it is submitted that the original specification provides an example of the term "predefined desired functionalities" in various location, such as, paragraphs 2 and 23. Similarly, one skilled in the art would understand how to define a desired function of the enzyme. A person of ordinary skilled in the art could not be formulating enzymes without some predefined (preconceived) goal (desired function). Accordingly, one with ordinary skill in the art would screen for predefined, desired functions when formulating the enzyme.

For example, paragraph 2 of the original specification states:

> "formulating a glucose oxidase enzyme possessing a certain desired property or properties, and, in particular embodiments, for formulating a glucose oxidase enzyme having peroxide-resistant characteristics for use, by way of example, in a sensing device."

As described in the above-quoted section of the original patent application, one example of the predefined desired functionality can be the property of "peroxide-resistance."

For example, paragraph 23 of the original specification states:

> "Embodiments of the invention are directed to processes for formulating a glucose oxidase enzyme with a particular desired property, such as, for example, an improved resistance to peroxide."

As described in the above-quoted section of the original patent application, an example of the predefined desired functionality is the desired property of "resistance to peroxide." However, a person of ordinary skill in the art would not be formulating enzymes without some predefined (preconceived) goal (desired function).

In view of the foregoing, it is respectfully submitted that pending claims 7-10 and 11-18 are in compliance with 35 U.S.C. 112, second paragraph.

2. Appeal Of Rejection Of Claims 1-3 and 7-8 Under 35 U.S.C. 103(a)

Claims 1-3 and 7-8 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Valdes et al., Hatzinikolaou et al., and Stemmer. This rejection is respectfully traversed. Applicant requests that the rejection be reversed and the rejected claims allowed over Valdes et al., Hatzinikolaou et al., and Stemmer, in view of the following remarks.

Claim 1 recites a method for formulating an enzyme that is not disclosed by either Valdes et al., Hatzinikolaou et al., and Stemmer or a combination thereof. For example, the method of claim 1 recites, among other features:

> "obtaining an organism with a glucose oxidase gene;
> growing multiple colonies of the organism;
> altering the environment of the colonies; and
> screening the colonies to identify colonies with active glucose oxidase after altering the environment of the colonies."

Claim 1 recites several actions that, together, form the claimed method, where not any one or a combination of the above-cited references describes the combination of actions recited in claim 1. The cited references fail to teach, suggest or render predictable selecting pieces of the disclosed processes and combining them in the fashion that the Examiner suggests. Instead, the references, themselves, as well as other references of record teach a direction away from the present invention.

The mass of evidence of record in the application suggests that those skilled in the art were taking a direction that was completely different from that of the claimed invention. While the Examiner raises arguments as to obviousness to combine various parts of the cited references, none of the evidence of record supports the Examiner's proposal to select and combine portions of the various references. To the contrary, a number of references of record (including the primary reference relied upon by the Examiner) teach a direction different than the claimed invention and would lead one of ordinary skill in the art away from the claimed invention. Without the present disclosure as a guide, one of ordinary skill in the art would not have found it obvious to combine the above-cited references as suggested by the Examiner.

As described in more detail below:

a. The prior art of record does not teach or suggest or render predictable the claimed invention;

b. None of the prior art of record provide any teaching or suggestion or render predictable the combination of the Valdes et al, Hatzinikolaou et al. and Stemmer, as proposed by the Examiner, and the mass of evidence of record shows that the prior art teaches away from the claimed invention; and

c. Each of dependent claims 2-3 and 7-8 recites further features that distinguish those claims from the prior art. Accordingly, those claims do not stand or fall with claim 1.

a. The Rejection Is Improper Because The Prior Art Does Not Teach Or Suggest Or Render Predictable The Claimed Invention.

In particular, neither Valdes et al. nor Hatzinikolaou et al. nor Stemmer describe formulating a glucose oxidase enzyme by growing multiple colonies of a organism, altering the environment of the colonies, and screening the colonies to identify colonies with active glucose oxidase to make them resistant to peroxide degradation. Moreover, one of ordinary skill in the art would not have been led by the prior art of record to alter the environment of the colonies, much screen for desired peroxide resistance properties. Such procedures would have been a drastic departure from the state of the art and, without the benefit of the present specification as a guide, would not have been obvious to one of ordinary skill in the art.

The Examiner argues that Valdes et al. teaches that glucose oxidase in glucose sensors degrade over time due to hydrogen peroxide. The Examiner acknowledges that Valdes et al. do not teach a method of producing and formulating active glucose oxidase from colonies grown in the presence of peroxide. (Final Office Action of January 16, 2007, pg. 5, ll. 7-10.) As discussed in more detail below, instead, Valdes et al. teach addressing peroxide degradation by adding a chemical catalase or by attaching an immobilized enzyme to a support that deactivates hydrogen peroxide. (Valdes, pg. 375, Left Column, ll. 6-18)

The Examiner refers to Valdes et al.'s statement than to ensure longer sensor functionality, instead of replacing a degraded glucose oxidase sensor enzyme with a fresh enzyme is "to simply prevent the degradation of the enzyme" (Final Office Action of January 16, 2007, pg. 4, l. 15 to pg. 5 l. 4., citing Valdes et al., pg. 375, ll. 2-5). The Examiner attempts to use that statement out of context, as a springboard to imply that Valdes et al. would have suggested a process involving altering the environment of the colonies of glucose oxidase organism and screening colonies in the manner recited in the present claims. However, Valdes et al. immediately follow the above statement with a description of the use of chemical additives as the so-called "better options." Accordingly, Valdes et al. teach a specific direction (use of chemical additives) that departs from the then-conventional process of replacing a degraded enzyme with a fresh enzyme.

Valdes et al. is not the only reference of record that teaches that the direction taken by those skilled in the art was to use chemical additives. Indeed, other references of record similarly teach that direction of the art (e.g., U.S. Patent No. 6,689,265 to Heller et al.). Neither Valdes et al., nor any prior art of record that relates to peroxide degradation of glucose oxidase, describe or suggest altering the environment of the glucose oxidase colonies and screening the colonies for peroxide resistant properties for addressing peroxide degradation of glucose oxidase. Instead, as described in more detail below, Valdes et al. and other references of record show that the direction taken by those skilled in the art was away from the method of the presently claimed invention.

Because of this lack of disclosure in Valdes et al., the Examiner attempts to select pieces of each of the Hatzinikolaou et al. and Stemmer. However, none of those references teach formulating a glucose oxidase enzyme by altering the environment of the glucose oxidase colonies to make them resistant to peroxide degradation.

For example, as noted above, claim 1 recites a method for formulating an enzyme that includes, among other features, "obtaining an organism with a glucose oxidase gene" and "growing multiple colonies of the organism." The Examiner stated that Stemmer discloses a method of producing mutant enzymes by obtaining a library of genes of interest and creating a library of mutated genes by multiple cycles. (Final Office Action dated January 16, 2007, pg. 5, ll. 13-15.)

However, Stemmer provides no teaching or suggestion of obtaining an organism with a glucose oxidase gene or altering the environment of the glucose oxidase colonies. Stemmer describes common DNA mutation techniques, some of which are noted in the present application (e.g., at page 8, ll. 21-23). Indeed, even the present application refers to common ways to create a library of mutant genes, including Error-Prone Polymerase Chain Reaction ("Error-Prone PCR"), gene shuffling and other processes. However, it is well beyond the teaching of Stemmer or those common mutation processes to use such techniques to grow multiple colonies of glucose oxidase containing organism or to alter the environment of the colonies and then to screen

colonies for desired peroxide resistant properties in accordance with the presently claimed method. Stemmer's description of mutating DNA for the production of nucleic acid fragments or polynucleotides encoding mutant proteins, provides no suggestion or motivation to create a colonies of glucose oxidase organism, and altering their environments and screening the colonies for desired peroxide resistant qualities.

Subsequently, the Examiner stated that Hatzinikolaou et al. discloses a library of glucose oxidase genes known in the art. However, Hatzinikolaou et al. describe isolating and characterizing a new synthesized glucose oxidase for purposes of conducting certain specified analyses (described on pages 373 and 374 of the Hatzinikolaou et al. reference), none of which relate to resistance to hydrogen peroxide (claim 7 and 8), or altering the environment of the glucose oxidase organism colonies (claim 1).

While gene libraries have been employed by those skilled in the art for gene analysis, Hatzinikolaou et al. provide no suggestion to use such libraries in the formulation of an enzyme by directed evolution. Hatzinikolaou et al. teaching of using gene libraries to analyze characteristics of a gene provides no motivation or suggestion or render predictable to do anything more than to analyze the specific new synthesized glucose oxidase for the specific characteristics described on pages 373 and 374 of that reference. The Examiner has picked only the feature of forming a gene library of glucose oxidase gene from Hatzinikolaou et al.'s overall process and seeks to combine that teaching with Stemmer (and other references cited in the rejection).

However, Hatzinikolaou et al.'s purpose of forming a library of a new simulated glucose oxidase (for analyzing the characteristics of the new simulated glucose oxidase described in that reference) would have no applicable purpose in any mutation process described by Stemmer. Once Hatzinikolaou et al. obtains and isolates a sample of the new glucose oxidase, Hatzinikolaou et al. conducts analysis on the isolated sample. Mutating the sample according to Stemmer would not allow Hatzinikolaou et al. to analyze the characteristics of the simulated glucose oxidase (as the mutations could effect the detection of characteristics under analysis).

Accordingly, it would not have been obvious to look to Hatzinikolaou et al. as a teaching of growing multiple colonies of a glucose oxidase organism, altering the environment to the colonies and screening the colonies to identify colonies with peroxide resistant properties. The Examiner's suggestion to combine Hatzinikolaou et al. with Stemmer is, therefore, respectfully traversed. Moreover, Hatzinikolaou et al. does not disclose or suggest screening colonies for active glucose oxidase predefined, desired peroxide resistant properties.

Because Stemmer does not relate to obtaining a multiple colonies of glucose oxidase organism or a altering the environment of the colonies, it follows that Stemmer also does not describe screening colonies by determining whether the colonies contain active glucose oxidase and determining whether the colonies have predefined, desired peroxide resistant properties. (claims 7 and 8) Also, Hatzinikolaou et al. provide no teaching or suggestion of screening colonies for active glucose oxidase having a desired peroxide resistant property. While the combination of those references is traversed for reasons noted above, no combination of those references could lead to screening colonies for active glucose oxidase having a desired peroxide resistant property because neither of those references, describe such a feature. Moreover, as noted above, because Valdes et al. also do not teach to screening colonies for active glucose oxidase and, instead, teach a very different direction (addition of chemicals to reduce peroxide degradation), the Examiner's suggestion to combine Valdes et al. with Stemmer and Hatzinikolaou et al. is traversed and would not lead to the present invention.

None of the cited references describes or suggests creating colonies of glucose oxidase containing organism, altering the environment of the colonies and then screening colonies for active glucose oxidase having a desired peroxide resistant properties. Accordingly, the combination of the references (as suggested by the Examiner) could not result in the claimed invention. The rejection of claims 1-3 and 7-8 under 35 U.S.C. 103(a) is, therefore, respectfully traversed and should be reversed.

b. The Rejection Is Improper Because Prior Art Provides No Motivation To Combine And Teaches Away From The Combination Suggested By The Examiner.

Because of the above-noted lack of disclosure in Valdes et al. (of growing multiple colonies of the glucose oxidase organism and screening the colonies to identify colonies with active glucose oxidase after altering the environment of the colonies), the Examiner attempts to select pieces of each of the Stemmer, Hatzinikolaou et al., and combine those with the Valdes et al. reference.

None of the Valdes et al, Hatzinikolaou et al. or Stemmer, references provide any teaching of creating multiple colonies of glucose oxidase containing organism, altering the environment of the colonies and screening colonies for active glucose oxidase with predefined, desired peroxide resistant properties, or methods of formulating a glucose oxidase enzyme with peroxide resistance.

Indeed, Valdes et al. teach away from such methods by, instead, referring to conventional procedures (using additives for deactivating or destroying hydrogen peroxide) and, thus, teach away from such a method, as follows:

> "To prohibit the H_2O_2 from degrading the GOD enzyme, it has been proposed that catalase be coimmobilized with GOD ... The addition of catalase in either the GOD itself, or to the incubating solution has resulted in a slower deactivation of the GOD enzyme ... A long term remedy of the degradation of GOD by H_2O_2 could be the immobilization and attachment of the enzyme to a support that deactivates H_2O_2, as it is being produced. Such as study was conducted by Cho[2], using the peroxide decomposition catalyst, activated carbon. In a study conducted by Carter[19], the best results were obtained with activated carbon, impregnated with ruthenium. This combination was able to destroy hydrogen peroxide and stabilized the enzyme." (Valdes et al., pg. 375, col. 1, l.18 to col. 2, l. 6.)

Not only does Valdes et al. fail to teach or suggest to alter the environment of glucose oxidase colonies or to screen the colonies to identify colonies with active glucose, but, in the above-quoted statement, Valdes et al. further teaches to use other, very different procedures

(conventional in the art) to address degradation effects of peroxide on glucose oxidase. Thus, the Valdes et al. reference shows that the direction taken by those most skilled in the art involved employing materials, additives, or the like that deactivate peroxide.

Additional prior art of record also describes conventional "additive" processes for removing or neutralizing peroxide such as by adding an antioxidant or peroxidase to the glucose oxidase to break down peroxide or by coating the glucose oxidase enzyme with a protective coating, including U.S. Patent No. 6,689,265 to Heller et al. Those prior art references further emphasize that the direction taken by those skilled in the art for addressing the peroxide degradation of glucose oxidase is wholly different from the direction of the present invention. In U.S. Patent No. 6,689,265 to Heller et al., a peroxide generating enzyme may include a sufficiently thick, natural, electrically insulating protein or glycoprotein layer. (See column 6, lines 59-67 of the Heller et al. patent) Heller et al. also disclose an alternative embodiment in which a peroxide generating enzyme is immobilized in a non-conducting inorganic or organic polymeric matrix. (See column 7, lines 3-11 of the Heller et al. patent) Also, Heller et al. describe a first layer enzyme 11 (peroxidase) that reduces peroxide generated from a second layer (glucose oxidase layer) 13.

Thus, the Heller et al. patent shows that the direction taken by those skilled in the art is to provide additives or complex multi-layer sensor structures to remove hydrogen peroxide. These references, in addition to Valdes et al.'s express references to conventional uses of additives, show that those skilled in the art were not considering growing, altering and screening colonies for peroxide resistance glucose oxidase organism, but instead were attempting to address the peroxide production issue by removing or neutralizing peroxide with additives (not by altering the glucose oxidase). The state and direction of the prior art, as evidenced by Valdes et al. and Heller et al., was a wholly different direction than that taken by the present Applicants (including altering the environment of the glucose oxidase colonies, screen the colonies to identify colonies with active glucose oxidase after altering the environment of the colonies (claim 1) and testing the colonies for predefined desired peroxide resistant functionality (claim 7)). Accordingly, the

mass of evidence of record (including the primary reference relied upon by the Examiner) teaches one skilled in the art taking a direction different from (and away from) the present invention.

Without the present disclosure as a guide, one of ordinary skill in the art would not have found Valdes et al.'s discussion of the degradation of glucose oxidase as a prompt or suggestion to employ a DNA mutation process as described Stemmer. Instead, as noted above, one of ordinary skill in the art would have looked to conventional manners of removing peroxide, such as additives for removing or neutralizing peroxide. Accordingly, the rejection of 1-3 and 7-8 under 35 U.S.C. 103(a) is further respectfully traversed.

The fact that the primary reference (Valdes et al.) teach away from the clamed invention and the combination suggested by the Examiner, shows that a *prima facie* case of obviousness has not been raised. Numerous Federal Circuit decisions recognize that an invention will not be deemed obvious in a patent law sense when one or more prior art references "teach away" from the invention. For example, the Federal Circuit stated "as a useful general rule, that references that teach away cannot serve to create a prima facie case of obviousness." *McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1354, 60 USPQ2d 1001 (Fed. Cir. 2001).

Furthermore, "an applicant may rebut a *prima facie* case of obviousness by showing that the prior art teaches away from the claimed invention in any material respect." *In re Peterson*, 315 F.3d 1325, 1331, 65 USPQ2d 1379 (Fed. Cir. 2003). Also see, *Gillette Co. v. S.C. Johnson & Sons, Inc.*, 919 F.2d 720, 724, 16 USPQ2d 1923, 1927 (Fed. Cir. 1990) (the closest prior art reference "would likely discourage the art worker from attempting the substitution suggested by [the inventor/patentee]") and *Singh v. Brake*, 317 F.3d 1334, 1346, 65 USPQ2d 1641 (Fed. Cir. 2003)("whether or not a reference 'teaches away' from a claimed invention" is "relevant in determining whether or not a claimed invention would have been obvious").

When patentability turns on the question of obviousness, the search for and analysis of the prior art includes evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the references relied on as evidence of obviousness.

(underline added for emphasis.) *See, e.g., McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1351—52, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001) ("the central question is whether there is reason to combine [the] references," a question of fact drawing on the *Graham* factors).

Conclusory statements that prior art references provide motivation to combine, or statements of motivation derived from the Applicant's own specification, are not sufficient to set forth a *prima facie* case of obviousness. "The factual inquiry whether to combine references must be thorough and searching." *Id.* It must be based on objective evidence of record. This precedent has been reinforced in myriad decisions. *See, e.g., Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1124— 25, 56 USPQ2d 1456, 1459 (Fed. Cir. 2000) ("a showing of a suggestion, teaching, or motivation to combine the prior art references is an 'essential component of an obviousness holding'") (quoting *C.R. Bard. Inc., v. M3* Systems, *Inc.*, 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed. Cir. 1998)); *In re Dembiczak,* 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) ("Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references."); *In re Dance,* 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998) (there must be some motivation, suggestion, or teaching of the desirability of making the specific combination that was made by the applicant); *In re Fine,* 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988) ("'teachings of references can be combined *only* if there is some suggestion or incentive to do so.') (emphasis in original) (quoting *ACS Hosp.* Sys., *Inc. 'v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)).

As noted above, the Examiner has not shown any motivation or suggestion in the prior art that would have led one skilled in the art to alter the environment of the colonies and screen for peroxide resistant properties. In fact, Valdes et al and other prior art of record show that altering the environment and screening process would have been a drastic diversion from the direction taken by those most skilled in the prior art.

The legal authority expresses the requirement for a showing of specificity in the prior art of motivation to select components to combine. *See, e.g., In re Kotzab,* 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000) ("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed"); *In re Rouffet,* 149 F.3d 1350, 1359, 47 USPQ2d 1453, 1459 (Fed. Cir. 1998) ("even when the level of skill in the art is high, the Board must identify specifically the principle, known to one of ordinary skill, that suggests the claimed combination. In other words, the Board must explain the reasons one of ordinary skill in the art would have been motivated to select the references and to combine them to render the claimed invention obvious."); *In re Fritch,* 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992) (the examiner can satisfy the burden of showing obviousness of the combination "only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references").

While the Valdes et al., Stemmer and Hatzinikolaou et al. references, themselves, provide no motivation or suggestion, the Examiner argues that one of ordinary skill in the art would have been motivated to do so in order to generate active glucose oxidase that is resistant to peroxide. (Final Office Action dated January 16, 2007, pg. 6, ll. 4-10.) While this is the likely conclusion, after reading the present disclosure as a guide, the references of record actually teach to do something very different (add chemicals) to reduce peroxide degradation of glucose oxidase. Thus, the mass of evidence of record shows that the motivation provided by the cited references would have been to reduce peroxide degradation by adding chemicals as taught by Valdes et al., U.S. Patent No. 6,689,265 to Heller et al.

In addition, the Examiner argues that one of ordinary skill in the art would have had a "reasonable expectation of success." However, without the present disclosure as a guide, one of ordinary skill in the art would not have selected altering the environment of glucose oxidase organism colonies, screening the colonies, purifying, isolating and measuring processes to

modify Valdes et al.'s disclosed solution to peroxide degradation of glucose oxidase. Valdes et al. teaches solutions to the peroxide degradation problem (by using chemical additives) and would have led one skilled in the art in the direction of those solutions. Stemmer does not mention glucose oxidase anywhere in its disclosure. Hatzinikolaou et al. fails to provide any motivation or suggest any relation to altering the environment of the glucose oxidase organism colonies or of addressing peroxide degradation of glucose oxidase. Moreover, the whole purpose of Hatzinikolaou et al. (to analyze a specific new simulated glucose oxidase) is not consistent with Stemmer's method of DNA reassembly or production of nucleic acid fragments or polynucleotides encoding mutant proteins.

The Examiner's conclusory statements of suggestion to combine and the Examiner's argument of "reasonable expectation of success" fail to address the significant issue of why one skilled in the art would have been motivated to select a process as described by Stemmer, to change the direction taken by those most skilled in the prior art as described by Valdes et al. The Examiner's argument that a "reasonable expectation of success" would have motivated the combination, is contrary to the express teachings of the prior art. The prior art teaches that those most skilled in the art were taking a wholly different direction to address peroxide degradation of glucose oxidase and, thus, would have found it unreasonable (not reasonable) to change the course of direction from that of the state of the art.

More specifically, Valdes et al. refer to completely different directions taken by those most skilled in the art, whereby the glucose oxidase enzyme is immobilized and attached to a support that deactivates peroxide. "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, ... would be led in a direction divergent from the path that was taken by the applicant." *Tec Air, Inc. v. Denso Mfg. Mich. Inc.*, 192 F.3d 1353, 1360, 52 USPQ2d 1294, 1298 (Fed. Cir. 1999). Valdes et al., directly refers the reader to conventional methods of addressing peroxide degradation of glucose oxidase that employ additives for destroying or neutralizing peroxide (which is quite different from creating multiple colonies, altering the environment and screening for desired peroxide resistant properties).

Because the Examiner has not shown any motivation or suggestion in the prior art that would have led one skilled in the art to select Stemmer's mutation process and materially change the direction taught by the Valdes et al. reference, the Examiner has not raised a *prima facie* case of obviousness. Therefore, the rejection of 1-3, 7 and 8 under 35 U.S.C. 103(a) is respectfully traversed.

c. Each of dependent claims 2-3, 7 and 8 recite further features that distinguish those claims from the prior art.

Each of dependent claims 2-3, 7 and 8 recite further featured that distinguish those claims from the prior art. In particular, each of those claims recites features relating to altering the environment of the colonies and screening the colonies to identify colonies with active glucose oxidase. As described above, neither Valdes et al., nor Hatzinikolaou et al., nor Stemmer references describe or suggest or render predictable altering the environment of the colonies. In that regard those references also do no disclose or suggest the additional processing recited in dependent claims 2-3, 7 and 8, including:

1. "the organism is selected from a group consisting of *Aspergillus Niger, Penecillium funiculosum, Saccharomyes cervisiae, and Escherichia Coli*" (claim 2);

2. "altering the environment of the colonies comprises introducing peroxide to the colonies" (claim 3);

3. "testing the colonies with active glucose oxidase for a predefined, desired functionality after screening the colonies to identify colonies with active glucose oxidase" (claim 7); and

4. "continuing to alter the environments of the colonies until the colonies with active glucose oxidase are of a suitable number to proceed with testing the colonies with active glucose oxidase for the predefined, desired functionality" (claim 8).

Because neither Valdes et al. nor Hatzinikolaou et al. nor Stemmer describe or suggest altering the environment of the colonies; and screening the colonies to identify colonies with active glucose oxidase after altering the environment of the colonies, those reference also do not disclose or suggest the additional processing recited in dependent claims 2, 3, 7 and 8. The rejection of claims 2, 3, 7 and 8 is, therefore respectfully traversed and should be reversed.

3. Appeal of Rejection Of Claims 4-6 and 9-17 Under 35 U.S.C. 103(a)

Claims 4-6, 9-16 and 17 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Valdes et al., Stemmer and Hatzinikolau et al. as applied to claims 1-3 and 7-8 above, and further in view of Wagner and Aldrich Catalog. This rejection is respectfully traversed. Applicant requests that the rejection be reversed and the rejected claims allowed over Valdes et al., Stemmer, Hatzinikolau et al., Wagner and Aldrich Catalog, in view of the following remarks.

Claims 4-6 and 9-17 directly or indirectly depend from claim 1 and thus claims 4-6 and 9-17 are patentable over Valdes et al., Stemmer and Hatzinikolau et al. for at least the same reasons as discussed above for claim 1. As discussed below, neither Wagner nor Aldrich Catalog (singularly or in combination) address the above noted distinctions, between the claimed invention and Valdes et al., Stemmer and Hatzinikolau et al.

Claim 4-6 and 9-17 recite a methods of formulating an enzyme that is not disclosed by either Valdes et al., Hatzinikolaou et al., Stemmer, Wagner and Aldrich Catalog. For example, the claims 4-6 recite, among other features:

1. "screening the colonies to identify colonies with active glucose oxidase comprises employing a substance that changes color in the presence of active glucose oxidase" (claim 4);

2. "the substance is leuco-crystal-violet"; (claim 5); and

3. "screening the colonies to identify colonies with active glucose oxidase comprises checking for fluorescence" (claim 6).

Claims 9-17 recite a methods of formulating an enzyme, among other features:

1. "testing the colonies with active glucose oxidase for the predefined, desired functionality comprises employing glucose oxidase from the colonies in sensors" (claim 9);

2. "testing the colonies further comprises: extracting glucose oxidase from the colonies; immobilizing the glucose oxidase after extracting the glucose oxidase from the colonies; placing the immobilized glucose oxidase in a sensor; and testing the sensor" (claim 10);

3. "extracting glucose oxidase from the colonies comprises employing an ionic column to extract glucose oxidase from the colonies" (claim 11);

4. "extracting glucose oxidase from the colonies comprises: removing the glucose oxidase from the colonies; purifying the glucose oxidase; and characterizing the glucose oxidase" (claim 12);

5. "removing the glucose oxidase from the colonies comprises grinding the colonies in a homogenizer into cell components" (claim 13);

6. "removing the glucose oxidase from the colonies further comprises fractionating the cell components employing centrifugation and differential solubility after grinding the colonies in a homogenizer" (claim 14);

10. "removing the glucose oxidase from the colonies comprises disrupting the colonies into cell components via sonication" (claim 15);

11. "removing the glucose oxidase from the colonies further comprises fractionating the cell components employing centrifugation and differential solubility after disrupting the colonies via sonication; (claim 16); and

12. "purifying the glucose oxidase comprises purifying the glucose oxidase by employing chromatography methods" (claim 17).

The methods recited in claims 4-6 and 9-17 recite several actions that, together, form the claimed method, where none of the above-cited references (singularly or in combination) describe the combination of actions recited in claims 4-16 and 9-17. The cited references fail to teach, suggest, motivate or render predictable selecting pieces of the disclosed processes and combining them in the fashion that the Examiner suggests. Instead, the references, themselves, as well as other references of record teach a direction away from the present invention.

The mass of evidence of record in the application suggests that those skilled in the art were taking a direction that was completely different from that of the claimed invention. While the Examiner raises arguments as to obviousness to combine various parts of the cited references, none of the evidence of record supports the Examiner's proposal to select and combination portions of the various references. To the contrary, a number of references of record (including the primary reference relied upon by the Examiner) teach a direction different than the claimed invention and would lead one of ordinary skill in the art away from the claimed invention. Without the present disclosure as a guide, one of ordinary skill in the art would not have found it obvious to combine the above-cited references as suggested by the Examiner.

As described in more details below:

a. The prior art of record does not teach or suggest or render predictable the claimed invention.

b. None of the prior art of record provide any teaching or suggestion for the combination of the Valdes et al. Stemmer, Hatzinikolau et al., Wagner et al. and Aldrich Catalog as proposed by the Examiner, and mass of evidence of record shows that the prior art teaches away from the claimed invention.

a. The Rejection Is Improper Because The Prior Art Does Not Teach Or Suggest The Claimed Invention.

In particular, claims 4-6 and 9-17 depend directly or indirectly from claim 1. As discussed above with regard to claim 1, Valdes et al., Hatzinikolau et al. and Stemmer do not teach, suggest or render predictable the features recited in claim 1. Similarly, claims 4-6 and 9-17 recite further features that are not rendered predictable by Valdes et al., Hatzinikolau et al. and Stemmer. The Examiner cites the Wagner and the Aldrich Catalog as teaching, suggesting or rendering predictable the features recited in claims 4-6 and 9-17. This rejection is respectfully traversed.

In particular, the Examiner cited the Wagner reference for disclosing a method of determining glucose oxidase activity in a sensor by measuring fluorescence. Wagner's method is used for continuous monitoring of glucose in a body fluid. Wagner neither describes nor suggests formulating an enzyme, much less growing multiple colonies of the glucose oxidase containing organism, screening colonies for predefined, desired properties by determining whether the colonies contain active glucose oxidase or determining whether the colonies have predefined desired peroxide resistant properties. Accordingly, Wagner does not address the above-noted distinctions between the claimed invention and the Valdes et al., Stemmer and Hatzinikolaou et al. references. Thus, the combination of Wagner with those other references (as suggested by the Examiner) could not result in the claimed invention.

The Examiner cited the Aldrich Catalog as describing Leuco-crystal violet dyes as common fluorescent dyes. The cited portion of the Aldrich Catalog neither describes nor suggests formulating an enzyme, much less growing colonies of glucose oxidase organism for predefined, desired properties by determining whether the colonies contain active glucose oxidase or determining whether the colonies have predefined desired peroxide resistant properties. Accordingly, the cited portion of the Aldrich Catalog does not address the above-noted distinctions between the claimed invention and the Valdes et al., Stemmer, Hatzinikolaou et al. and Wagner references. Thus, the combination of the cited portion of the Aldrich Catalog with those other references (as suggested by the Examiner) could not result in the claimed invention.

None of the Valdes et al, or Stemmer, Hatzinikolaou et al., Wagner et al. and Aldrich Catalog references provide any teaching, suggestion or render predictable growing multiple colonies of glucose oxidase containing organism, altering the environment of the colonies and screening colonies for active glucose oxidase with predefined, desired peroxide resistant properties, or methods of formulating a glucose oxidase enzyme with peroxide resistance properties. Accordingly, the combination of the references (as suggested by the Examiner) could not result in the claimed invention. The rejection of claims 4-6 and 9-17 under 35 U.S.C. 103(a) is, therefore, respectfully traversed and should be reversed.

b. The Rejection Is Improper Because Prior Art Provides No Motivation To Combine And Teaches Away From The Combination Suggested By The Examiner.

In addition the rejection based on an attempt to combine Valdes et al. with other references relating to gene mutation, the rejection under 35 U.S.C. 103 is improper because of a lack of motivation (without the present disclosure as a guide).

As noted above, the Examiner has acknowledged that "Valdes et al. does not teach a method of producing mutant glucose oxidase that is resistant to degradation from peroxide." (Final Office Action dated January 16, 2007, pg. 8, l. 20 – pg. 9, l. 2.) Accordingly, the Examiner argues it would have been obvious to combine Stemmer, Hatzinikolaou et al., Wagner et al. and the Aldrich Catalog references with the Valdes process.

However, the Examiner cites no teaching in either the Valdes et al. reference or the Stemmer, Hatzinikolaou et al., Wagner et al. or Aldrich Catalog references (or any other prior art) for incubating colonies of glucose oxidase with hydrogen peroxide. In fact, neither Valdes et al., Stemmer, Hatzinikolaou et al., Wagner et al. nor the Aldrich Catalog references provide any motivation or suggestion for creating colonies of glucose oxidase organisms, altering the environment of the colonies, screening colonies for active glucose oxidase and desired peroxide resistant properties. Indeed, Valdes et al. teach away from such methods by, instead, referring to

conventional procedures (using additives for deactivating or destroying hydrogen peroxide and, thus, teach away from such a method, as follows:

> "To prohibit the H_2O_2 from degrading the GOD enzyme, it has been proposed that catalase be coimmobilized with GOD ... The addition of catalase in either the GOD itself, or to the incubating solution has resulted in a slower deactivation of the GOD enzyme ... A long term remedy of the degradation of GOD by H_2O_2 could be the immobilization and attachment of the enzyme to a support that deactivates H_2O_2, as it is being produced. Such as study was conducted by Cho[3], using the peroxide decomposition catalyst, activated carbon. In a study conducted by Carter[19], the best results were obtained with activated carbon, impregnated with ruthenium. This combination was able to destroy hydrogen peroxide and stabilized the enzyme." (Valdes et al., pg. 375, col. 1, l.18 to col. 2, l. 6.)

Not only does Valdes et al. fail to teach or suggest to alter the environment and screen glucose oxidase organism for peroxide resistance properties, but, in the above-quoted statement, Valdes et al. further teaches to use other, very different procedures (conventional in the art) to address degradation effects of peroxide on glucose oxidase. Thus, the Valdes et al. reference shows that the direction taken by those most skilled in the art involved employing materials, additives, or the like that deactivate peroxide.

None of the cited references describes or suggests creating colonies of glucose oxidase containing organism, altering the environment of the colonies and then screening colonies for active glucose oxidase having a desired peroxide resistant property. Accordingly, the combination of the references (as suggested by the Examiner) could not result in the claimed invention. The rejection of claims 4-6 and 9-17 under 35 U.S.C. 103(a) is, therefore, respectfully traversed and should be reversed.

Additional prior art of record also describes conventional "additive" processes for removing or neutralizing peroxide such as by adding an antioxidant or peroxidase to the glucose oxidase to break down peroxide or by coating the glucose oxidase enzyme with a protective coating, including U.S. Patent No. 6,689,265 to Heller et al. The Heller et al. reference further emphasize that the direction taken by those skilled in the art for addressing the peroxide

degradation of glucose oxidase is wholly different from the direction of the present invention. In U.S. Patent No. 6,689,265 to Heller et al., a peroxide generating enzyme may include a sufficiently thick, natural, electrically insulating protein or glycoprotein layer. (See column 6, lines 59-67 of the Heller et al. patent) Heller et al. also disclose an alternative embodiment in which a peroxide generating enzyme is immobilized in a non-conducting inorganic or organic polymeric matrix. (See column 7, lines 3-11 of the Heller et al. patent) Also, Heller et al. describe a first layer enzyme 11 (peroxidase) that reduces peroxide generated from a second layer (glucose oxidase layer) 13.

The Wagner reference does not address the above-noted distinctions between the claims and the Valdes et al., Stemmer and Hatzinikolaou references. Indeed, the Wagner reference was cited, according to the Examiner, for disclosing a method of determining glucose oxidase activity via a sensor by measuring fluorescence emission from a dye, wherein oxidation of glucose by active glucose oxidase reduces the florescence emission. However, Wagner does not teach or suggest formulating a glucose oxidase enzyme by altering the environment and screening glucose oxidases to make them resistant to peroxide degradation. Accordingly, the combination of Wagner with the above-discussed references (the Valdes et al., Stemmer and Hatzinikolaou references) would not lead to the presently claimed invention. The Aldrich Catalog also does not address the above-noted distinctions between the claims and the Valdes et al., Stemmer and Hatzinikolaou references.

Claims 4-6 and 9-17 were rejected under 35 U.S.C. 103(a) as being unpatentable over Valdes et al., Stemmer and Hatzinikolaou and further in view of Wagner and Aldrich catalog. The rejection of claims 4-6 and 9-17 is respectfully traversed, at least for reasons as discussed above with respect to parent claim 1. Wagner and the Aldrich catalog references do not address the above-noted distinctions between the claims and the Valdes et al., Stemmer and Hatzinikolaou references.

As noted above, the Examiner has not shown any motivation or suggestion in the prior art that would have led one skilled in the art to alter the environment of the colonies and screen for

peroxide resistant properties. In fact, Valdes et al and other prior art of record show that altering the environment and screening process would have been a drastic diversion from the direction taken by those most skilled in the prior art.

As noted above the legal authority expresses the requirement for a showing of specificity in the prior art of motivation to select components to combine. Because the Examiner has not shown any teaching, motivation, suggestion or rendered predictable that would have led one skilled in the art to select processes from et al., Hatzinikolaou et al., Stemmer, Wagner and Aldrich Catalog, the Examiner has not raised a *prima facie* case of obviousness. Therefore, the rejection of 4-6 and 9-17 under 35 U.S.C. 103(a) is respectfully traversed.

Conclusion

In view of the foregoing, it is respectfully submitted that claims 1-17 are in condition for allowance and the application should be allowed in its present form. In particular, it is respectfully submitted that the presently pending rejections of claims 1-17 are improper and should be reversed for reasons as discussed above. In that regard, each of claims 1-17 is in condition for allowance.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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VIII. CLAIMS APPENDIX

1. (Original) A method for formulating an enzyme comprising:

obtaining an organism with a glucose oxidase gene;

growing multiple colonies of the organism;

altering the environment of the colonies; and

screening the colonies to identify colonies with active glucose oxidase after altering the environment of the colonies.

2. (Previously Presented) A method for formulating an enzyme according to claim 1, wherein the organism is selected from a group consisting of *Aspergillus Niger, Penecillium funiculosum, Saccharomyces cerevisiae,* and *Escherichia Coli.*

3. (Original) A method for formulating an enzyme according to claim 1, wherein altering the environment of the colonies comprises introducing peroxide to the colonies.

4. (Original) A method for formulating an enzyme according to claim 1, wherein screening the colonies to identify colonies with active glucose oxidase comprises employing a substance that changes color in the presence of active glucose oxidase.

5. (Original) A method for formulating an enzyme according to claim 4, wherein the substance is leuco-crystal-violet.

6. (Previously Presented) A method for formulating an enzyme according to claim 1, wherein screening the colonies to identify colonies with active glucose oxidase comprises checking for fluorescence.

7. (Previously Presented) A method for formulating an enzyme according to claim 1, wherein the method further comprises testing the colonies with active glucose oxidase for a predefined, desired functionality after screening the colonies to identify colonies with active glucose oxidase.

8. (Previously Presented) A method for formulating an enzyme according to claim 7, wherein the method further comprises continuing to alter the environments of the colonies until the colonies with active glucose oxidase are of a suitable number to proceed with testing the colonies with active glucose oxidase for the predefined, desired functionality.

9. (Previously Presented) A method for formulating an enzyme according to claim 7, wherein testing the colonies with active glucose oxidase for the predefined, desired functionality comprises employing glucose oxidase from the colonies in sensors.

10. (Previously Presented) A method for formulating an enzyme according to claim 7, wherein testing the colonies with active glucose oxidase for the predefined, desired functionality comprises:

extracting glucose oxidase from the colonies;

immobilizing the glucose oxidase after extracting the glucose oxidase from the colonies;

placing the immobilized glucose oxidase in a sensor; and

testing the sensor.

11. (Original) A method for formulating an enzyme according to claim 10, wherein extracting glucose oxidase from the colonies comprises employing an ionic column to extract glucose oxidase from the colonies.

12. (Original) A method for formulating an enzyme according to claim 10, wherein extracting glucose oxidase from the colonies comprises:

removing the glucose oxidase from the colonies;

purifying the glucose oxidase; and

characterizing the glucose oxidase.

13. (Original) A method for formulating an enzyme according to claim 12, wherein removing the glucose oxidase from the colonies comprises grinding the colonies in a homogenizer into cell components.

14. (Original) A method for formulating an enzyme according to claim 13, wherein removing the glucose oxidase from the colonies further comprises fractionating the cell components employing centrifugation and differential solubility after grinding the colonies in a homogenizer.

15. (Original) A method for formulating an enzyme according to claim 12, wherein removing the glucose oxidase from the colonies comprises disrupting the colonies into cell components via sonication.

16. (Original) A method for formulating an enzyme according to claim 15, wherein removing the glucose oxidase from the colonies further comprises fractionating the cell components employing centrifugation and differential solubility after disrupting the colonies via sonication.

17. (Original) A method for formulating an enzyme according to claim 12, wherein purifying the glucose oxidase comprises purifying the glucose oxidase by employing chromatography methods.

18. (Withdrawn) An enzyme formulated according to the method of claim 1.

IX. EVIDENCE APPENDIX

None.

X. RELATED PROCEEDINGS APPENDIX

None.